CLONAL SPREAD OF MULTI-DRUG RESISTANT KLEBSIELLA PNEUMONIAE ISOLATES IN A LARGE TEACHING HOSPITAL IN THE UK

M. Naeem Khattak , Adam Fraise

Hospital Infection Research Laboratory, City Hospital NHS Trust
Birmingham, UK

ABSTRACT

Objective: *To study hospital-wide dissemination of Extended Spectrum Beta-Lactamase (ESBL) producing Klebsiella pneumonaie strains in a University Teaching Hospital in the West Midlands region of the UK.*

Material and Methods: *ESBL producing Klebsiella pneumonaie strains were isolated from patients admitted to the study hospital during a two months period. Two Klebsiella pneumonaie NCTC strains, 10896 and 9633 were used as controls. Biotyping profiles were determined by using API 20E system (API-BioMerieux, France) as per manufacturer's instructions. Initial susceptibility testing of the isolates was performed by the BSAC disc diffusion method on IsoSensitest agar (ISA; Oxoid, Basingstoke, UK). ESBL producing isolates were further tested by the BSAC broth dilution method using MIC breakpoint susceptibility tests. Isolates were grown for 24 hours at 37 oC in 5 ml of Brain Heart infusion (BHI; Oxoid, Basingstoke, UK). Restriction digestion of chromosomal DNA and Pulsed-field gel electrophoresis (PFGE) were carried out.*

Results: *The sixteen clinical isolates from the study hospital clustered into two API biotype profiles. Their antibiogram profile was similar except that eight isolates were resistant to ciprofloxacin in addition to other antibiotics tested. There was no apparent correlation between the API profile and antibiogram among these patients. Of the sixteen ESBL producing Klebsiella pneumoniae isolates from the study hospital, 9 were from urinary samples, 5 isolates were from sputum, and the remaining two strains were from blood cultures. For the 20 clinical isolates and two control strains examined, 6 Xba 1 restriction patterns were observed. The 16 clinical isolates from our hospital produced identical DNA profile. These were designated as type A. The four unrelated isolates from a different hospital produced two DNA types designated as type B and type C. The two control strains produced fragment patterns different to each other and to the remainder of the isolates. These were designated as type E and type F .*

Conclusion: *This study emphasizes the need for continued surveillance of ESBL producing enterobacteriacae. This will be helpful in monitoring antimicrobial resistance, and to guide intervention to minimize its occurrence.*

Keywords: *ESBL Producing Klebsiella Pneumoniae, Clonal Spread, Molecular Analysis*

INTRODUCTION

Extended Spectrum Beta-Lactamase (ESBL) producing organisms are becoming increasingly common among hospitalised patients[1]. Major risk factors for colonization or infection with ESBL-producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, instrumentation or catheterisation[2-4]. The most common ESBL-producers are Escherichia coli and Klebseilla species[5]. These contain multi-resistant plasmids that may be transmitted among other members of the enterobacteraicae, even within the same patients[6]. Apart from resistance to Beta-lactam antibiotics, ESBLs are frequently associated with resistance to other classes of antibiotics, including aminoglycosides and fluoroquinolones[7,8]. This poses an important challenge in clinical practice, since these organisms are a common cause of serious infections. This may have implications in terms of treatment and outcome, and infection control[9,10].

We report the hospital-wide dissemination of ESBL producing *Klebsiella pneumonaie* strains in a University Teaching Hospital in the West Midlands region of the UK. An increase in ESBL producing *Klebsiella pneumonaie* was observed over a 2 month period amongst the hospitalised

patients. This prompted us to analyse the clinical and molecular epidemiology of the isolates in these patients. The aim of the study was to determine whether it was a true outbreak as a result of the spread of a single strain from a single source, and to adopt measures to eradicate a potentially dangerous nosocomial pathogen. Conventional typing techniques applied to characterise *Klebsiella* species lack reproducibility, typeability and discrimination[11]. Hence, the technique of Pulsed-field gel electrophoresis (PFGE) was applied to delineate strains below species level. The same methods have been successfully applied to type *Klebsiella* outbreak in the past[12,13].

MATERIAL AND METHODS

Isolates:

Sixteen ESBL producing Klebsiella pneumonaie strains were isolated from patients admitted to the study hospital during a two months period. Four isolates recovered from patients in another geographically unrelated hospital were also included. The details of the isolates are given in Table I. Two *Klebsiella pneumonaie* NCTC strains, 10896 and 9633 were used as controls.

Biotyping:

Biotyping profiles were determined by using API 20E system (API-BioMerieux, France) as per manufacturer's instructions.

Antibiogram:

Initial susceptibility testing of the isolates was performed by the BSAC disc diffusion method[14] on IsoSensitest agar (ISA; Oxoid, Basingstoke, UK). ESBL producing isolates were further tested by the BSAC broth dilution method[15,16] using MIC breakpoint susceptibility tests. The resistance profile is presented in Table I.

Preparation of chromosomal DNA:

Isolates were grown for 24 hours at 37 °C in 5 ml of Brain Heart infusion (BHI; Oxoid, Basingstoke, UK). One ml of overnight broth for each isolate was transferred to an eppendorf tube. The cells were pelleted, and washed twice with

DETAILS OF THE ESBL PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES FROM THE SAME HOSPITAL, UNRELATED ISOLATES FROM A DIFFERENT HOSPITAL, AND CONTROL STRAINS.

Isolate No	Source of the isolate	Ward	API profile	*Antibiogram	DNA type
Isolates from the same hospital					
1	Urine	M8	5215772	AmoCxmAugCazTimTic	A
2	Urine	D11	5215772	AmoCxmAugCazTimTicCip	A
3	Urine	D47	5215772	AmoCxmAugCazTimTic	A
4	Urine	D46	5215772	AmoCxmAugCazTimTic	A
5	Urine	D16	5215772	AmoCxmAugCazTimTic	A
6	Sputum	D16	5215772	AmoCxmAuCazTimTicCip	A
7	Sputum	D11	5215772	AmoCxmAugCazTimTic	A
8	Sputum	D47	5215772	AmoCxmAugCzTimTicCip	A
9	Urine	GP	5215772	AmoCxmAugCazTimTicCip	A
10	Blood	D18	5215773	AmoCxmAugCazTimTicCip	A
11	Urine	D42	5215773	AmoCxmAugCazTimTicCip	A
12	Sputum	D47	5215773	AmoCxmAugCazTimTicCip	A
13	Sputum	D30	5215773	AmoCxmAugCazTimTic	A
15	Urine	D27	5215773	AmoCxmAugCazTimTicCip	A
16	Urine	D11	5215773	AmoCxmAugCazTimTic	A
Isolates from a different hospital					
17	Blood				B
18	Swab				C
19	Urine				C
20	Swab				B
NCTC strains used as controls					
21	NCTC10896				D
22	NCTC9633				E

*Antibiograms denoted as a resistance profile based on testing with Ampicillin(Amo), Cefuroxime(Cxm), Augmentin(Aug), Ceftazidime(Caz), Timentin(Tim), Ticarcillin(Tic), Ciprofloxacin(Cip).

Table I

1ml of TEN buffer (6 mM Tris-HCl pH 7.5, 100 mM EDTA pH 8.0 & 1M NaCl). An equal volume of 2% low gelling agarose (Bio-Rad, USA) was added, and the mixture dispensed into a 10-plug mould (Bio-Rad, USA). Plus were solidified at 4°C for 5min and then divided into four pieces with a clean coverslip. The plugs were incubated for 24 hours at 37°C in sterile screw capped bottles containing 3ml of lysis buffer (6mM Tris-HCl pH 8.0, 100 mM EDTA pH 8.0,1 M NaCl, 1 g/l lysozyme (Sigma) and 100 ul of 30% N-lauryl Sarcosine (Sigma). The plugs were further treated for 48 hours at 50°C in 2 ml of ESP solution (0.5 M EDTA pH 9.0, Proteinase K 1g/l, 100ul of 30% N-lauryl Sarcosine). The plugs were washed twice with 3ml of TE buffer (Tris-HCl pH 7.5, 1 mM EDTA pH 8.0) containing 1mM Phenylmethyl sulphonyl fluoride (PMSF). The plugs were further washed with three changes of TE buffer without PMSF and stored until use.

Restriction digestion of chromosomal DNA:

Two plugs of each strain were collected in an eppendorf tube and equilibrated in 300 ul of *Xba*1 (Sigma) buffer for 30 min on ice. Subsequently, 30 units of *Xba*1 enzyme added and digestion carried out for 24 hours at 37 °C. The reaction was stopped by the addition of 50 mM EDTA.

Pulsed-field gel electrophoresis (PFGE):

PFGE was performed using the CHEF DR-II system (Bio-Rad, Carolina, USA). Gels were made up of 1.0% agarose in 0.5% TBE buffer (Tris-HCl 10.3 gm, Boric acid 5.5gm, EDTA 0.93gm). The plugs were loaded into separate wells. Bacteriophage lambda DNA ladder (Sigma) was included in each gel as a molecular weight marker. Electrophoresis was performed with a ramped pulse time of 10-70 seconds for 24 hours at field strength of 6V/cm. Gels were stained with 25l of Ethidium bromide in distilled water for 30min and photographed.

RESULTS

The sixteen clinical isolates from the study hospital clustered into two API biotype profiles. Their antibiogram profile was similar except that eight isolates were resistant to ciprofloxacin in addition to other antibiotics tested (Table 1). There was no apparent correlation between the API profile and antibiogram among these patients. Of the sixteen ESBL producing *Klebsiella pneumoniae* isolates from the study hospital, 9 were from urinary samples, 5 isolates were from sputum, and the remaining two strains were from blood cultures. Details of the colonised or infected cases are presented in Table I. In order to analyse the relatedness of the isolates, *Xba*1 restriction enzyme was selected which recognises the tetra-nucleotide sequence CTAG in Gram-negative bacteria. All the isolates were typeable, and had excellent reproducibility as judged by visual comparison between gels run under identical conditions. The PFGE profiles were stable on repeat testing of the same isolate after subculture on three separate occasions. Cleavage of the *Klebsiella pneumoniae* DNA with *Xba*1 generated 13-16 large well differentiated DNA fragments per isolate in the size range of 48 to 530Kb.

For the 20 clinical isolates and two control strains examined, 6 Xba 1 restriction patterns were observed. The criteria of Tenover *et al*[17] (1995)

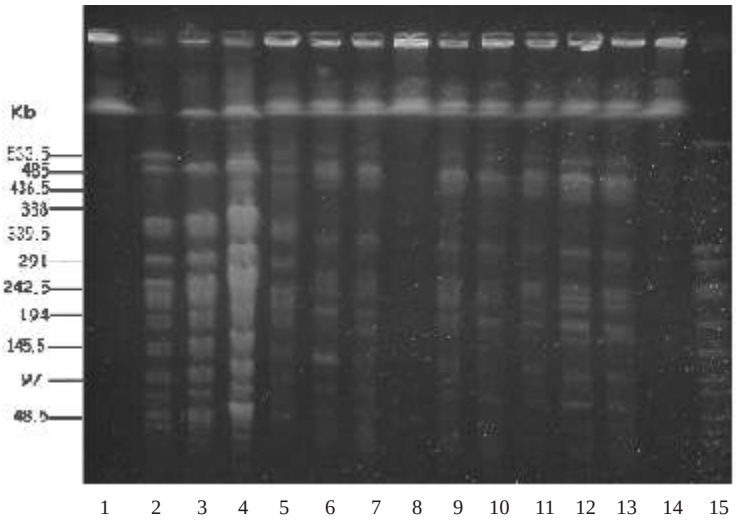

Figure 1. Details of DNA karyotype patterns of ESBL producing *Klebsiella pneumoniae* isolates; lanes 1, 8 & 14 (lambda DNA Ladder), lanes 2-5 (non-related isolates from a different hospital), lanes 6, 7 & 9 -13 (the study hospital isolates), and lane 15 (*S. aureus* NCTC strain 9366).

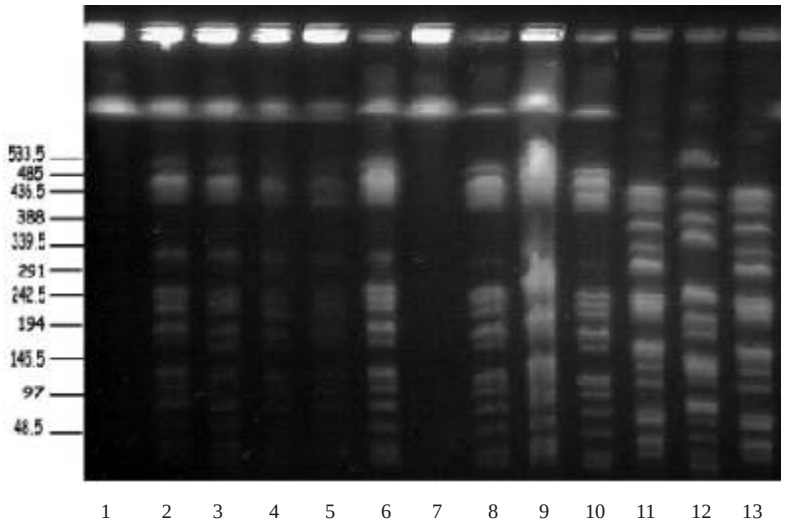

Figure 2. Details of DNA karyotype patterns of ESBL producing *Klebsiella pneumoniae* isolates; lanes 1, 7 & 14 (lambda DNA Ladder), lanes 2-6 & 8-10 (The study hospital isolates), lanes 11&13 (NCTC strains 10896) and lane 12 (NCTC strain 9633

were used to determine the relatedness of isolates. They state that genetically identical isolates produce identical DNA bands, and closely related isolates differ from the outbreak pattern by two to three bands. Possibly related strains differ by four to six bands, whereas unrelated isolates differ by seven or more band differences. The 16 clinical isolates from our hospital produced identical DNA profile. These were designated as type A (Figure 1, lanes 5-9 & 9-13 and Figure 2, lanes 2-6 & 8-13). The four unrelated isolates from a different hospital produced two DNA types designated as type B (Figure 1, lanes 2 & 5) and type C (Figure 1, lanes 3 & 4). The two control strains produced fragment patterns different to each other and to the remainder of the isolates. These were designated as type E (Figure 2; lanes 11 & 13) and type F (Figure 2; lane 12).

DISCUSSION

Epidemiological studies based on phenotypic characteristics such as API biotype profile and antibiotic susceptibility patterns may not accurately predict relatedness of the strains as shown in the present study. The biochemical system profile number of the API 20E clustered the 16 isolates from the same hospital into two biotypes. This did not correlate with the results of the PFGE typing. Similarly, the antibiogram profile grouped the isolates into two types based on their susceptibility pattern to ciprofloxacin (Table 1). Use of antibiotic resistance determinants may not be sufficiently discriminatory for outbreak investigation. Thus, these characteristics can not be necessarily relied on to discriminate strains of ESBL producing strains of *Klebsiella pneumoniae* within a hospital.

Analysis of the PFGE profiles using *Xba*1 restriction enzyme showed identical PFGE profiles in 16 isolates from the study hospital, although these did not appear to be epidemiologically related in terms of time and location. None of the patients was in the same ward at the time of isolation of the ESBL producing *Klebsiella pneumoniae*, but surprisingly all of these produced reproducibly the same DNA karyotype. The four geographically distant isolates produced different DNA types compared to the study isolates as was the case with the two control strains.

The majority of the ESBL producing *Klebsiella pneumoniae* were detected in urinary samples (9/16), whereas 5 isolates were recovered from sputum. The remaining two isolates were from blood cultures. Amongst the 15 admitted cases, 10 were not related in time and space. Two patients were admitted on the same ward, but their admissions were two weeks apart. Similarly, two other patients admitted to another ward were admitted four weeks apart. An isolate recovered from the respiratory specimen of a patient on two occasions (10 days apart) gave identical anti-biogram profile, but different API biotype.

The striking homogeneity of the DNA profile suggests the spread of a single clone of *Klebsiella pneumoniae* within the same hospital, although we were not able to establish any epidemiological evidence in terms of patients being admitted on the same wards at same time. However, these results emphasize the need for continued surveillance of ESBL producing enterobacteriacae. This will be helpful in monitoring antimicrobial resistance, and to guide intervention to minimize its occurrence. Good

infection control practices, especially good hand washing techniques are necessary to prevent spread of multi-drug resistant organisms. This combined with education of the staff and careful review of the nursing care practices was helpful in minimising further transmission of such cases in the study hospital.

REFERENCES

1. Nathisuwam S, Burgess DS, Lewis JS. Extended spectrum Beta-lactamases: epidemiology, detection and treatment. Pharmacotherapy 2001; 21(8): 920-8.
2. Medeiros AA. Nosocomial outbreaks of mutliresistant bacteria: extended-spectrum Beta-lactamases have arrived in North America. Ann Intern Med 1993; 119:353-8.
3. Schiappa DA, Hayden MK, Matushek MG, et al. Ceftazidime-resistant Klebsiella pneumoniae and Escherichia coli in blood stream infection: a case-control and molecular epidemiologic investuagation. J Infect Dis 1996; 174:539-6.
4. Jacoby GA. Editorial response: epidemiology of extended-spectrum Beta-lactamases. Clin Infect Dis 1998; 27: 81-3.
5. Philippon A, Labia R, Jacoby G. Extended-spectrum Beta-lactamases. Antimicrob Agents Chemother 1989; 33: 1131-6.
6. Sirot DL, Champs CD, Chanal C, Labia R, Darfeuille-Michaud A, Perrous R, Sirot J. Translocation of antibiotic resistance determinants including an extended-spectrum Beta-lactamses between conjugative plasmids of Klebsiella pneumoniae and Escherichia coli. Antimicrob Agents Chemother 1991; 35: 1576-81.
7. Itokazu GS, Quinn JP, Bell-Dixon C, Kahn FM, Weinstein RA. Antimicrobial resistance rate among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national post-marketing surveillance program. Clin Infect Dis 1996; 23:779-84.
8. Jacoby GA. Genetics of extended spectrum Beta-lactamases. Eur J Clin Infect Dis 1994; 13(S 1): 2-11.
9. Bush, K. Editorial; Is it important to identify extended-spectrum Beta-lactamase-producing isolates?. Eur J Clin Microbiol Infec Dis 1996; 15(5): 361-364.
10. Ariffin H, Navaratnam P, Mohamed M, et al. Ceftazidime-resistant Klebsiella pneumoniae blood stream infection in children with febrile neutropenia. Int J infec Dis 2000; 4: 21-5
11. Ayling-Smith B, Pitt TL. State of the art in typing Klebsiella species. J Hosp Infect 1990; 16: 287-95.
12. Weller TM, MacKenzie FM, Forbes KJ. Molecular epidemiology of a large outbreak of multiresistant Klebsiella pneumoniae. J Med Microbiol 1997; 46: 927-6.
13. Poh CL, Yap SC, Yeo M. Pulsed-field gel electrophoresis for differentiation of hospital isolates of Klebsiella pneumoniae. J Hosp Infect 1993; 24:123-8.
14. Andrews JM. BSAC standardized disc susceptibility testing method. J Antimicrob Chemother 2001; 48(S1): 43-57.
15. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001; 48(S1): 5-16.
16. MacGowan AP, Wise R. Establishing MIC breakpoints and the interpretation of in vitro susceptibility tests. J Antimicrob Chemother 2001; 48(S1): 17-28.
17. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction produced by Pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 1995; 33: 2233-9.

Address for Correspondence:
M. Naeem. Khattak
Hospital Infection Research Laboratory
City Hospital NHS Trust Dudley Road,
Birmingham B18 7QH, UK